VARIATIONS IN CELL SIZE DURING THE DEVELOPMENT OF THE SLIME MOLD, DICTYOSTELIUM DISCOIDEUM [1]

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In many organisms, especially multicellular ones, it is relatively difficult to obtain an estimate of individual cell sizes, but in the amoeboid slime mold *Dictyostelium discoideum* it is not only possible to do this with reasonable accuracy for one stage, but for most of the stages of development. In the life cycle of this slime mold (Raper, 1935, 1951; Bonner, 1944) the amoebae are first separate during their actively feeding stage, and later after a short period of fasting they aggregate to form sausage-shaped cell masses which migrate for variable periods of time. During this migration stage the anterior cells begin their differentiation into stalk cells and the posterior cells begin their differentiation into spores (Bonner, 1952) and then finally the migrating mass shoots up into the air on a delicate stalk made up of the anterior cells which now have become vacuolated and encased in a hard cellulose sheath, and at the tip of this stalk there is a globular spore mass made up of cells encased in elliptical spore capsules. The range of individual cell sizes of each of these stages (with the exception of the mature stalk cells which could not be measured accurately because of their irregular shape) was determined and it was found that changes in the stage of development as well as early signs of differentiation were all reflected in characteristic changes of individual cell sizes.

MATERIALS AND METHODS

The method of culture and the culture medium were the same as those used previously and the aggregating and migrating amoebae were prepared by centrifuging the vegetative amoebae free of bacteria and placing them on plain agar (Bonner, 1947). The cells at each stage were removed and placed in a drop of standard solution (NaCl, 0.60 gm.; KCl, 0.75 gm.; CaCl$_2$, 0.30 gm.; distilled H$_2$O, 1000 ml.) on a microscope slide. A No. 1 coverslip (22 × 22 mm.) was placed over the drop, but supported on two bits of coverslip so as to prevent the cells from being crushed. The diameters of the rounded, spherical cells were then measured with a 95 X, oil immersion objective and a Zeiss filar micrometer. In the case of the spores, which are elliptical in shape, both the long and the short axes were measured, and the spore diameter is expressed as the mean of these two values for each spore measured.

A number of tests were run to determine if the diameter of an individual cell remained constant when immersed in standard solution. It was found that during a period from 5 to 90 minutes after immersion the cell diameter did remain fairly constant, the small fluctuations being apparently caused by the activity of the contractile vacuole.

The accuracy is considered to be most reasonable for the vegetative amoebae and the spores. However, during the aggregation and migration stages a fair per cent of the cells either did not round up after being subjected to the salt solution, or they remained part of a mass of cells and were impossible to measure. Therefore, during these two stages, there might conceivably be a sizable error due to having selected only those cells that were spherical. In defense of this it can only be said that there was no apparent or striking size difference and that it is believed that if an error has been introduced by this procedure it is likely to be relatively small.
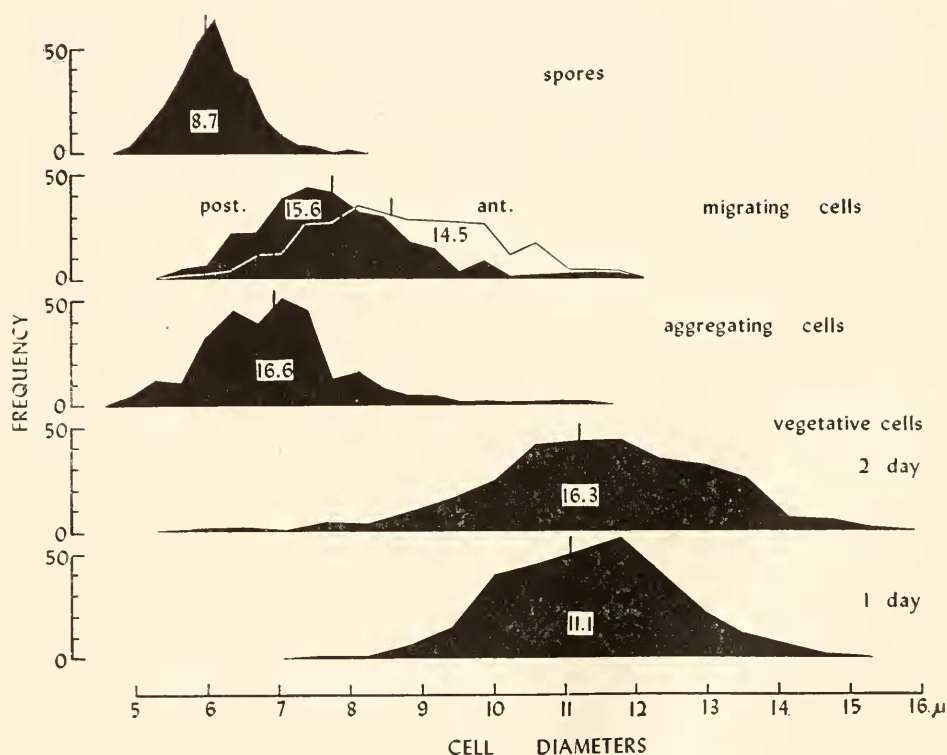

FIGURE 1. A graph in which the frequency is plotted against the cell diameter for various stages of development. Each curve consists of 300 observations. The vertical lines indicate the mean values. The numbers placed within the curves are the coefficients of variation in per cent.

RESULTS

The diameters of 300 cells were measured for the following stages of development: (1) vegetative amoebae approximately one day old (between 22.5 and 23.5 hrs.), (2) vegetative amoebae approximately two days old (between 41.5 and 46 hrs.), (3) aggregating amoebae, (4) and (5) the anterior and posterior cells at the beginning of the migration stage (3 to 4 mm. of migration) and (6) the mature spores. For each stage, this count of 300 was made on three separate

samples of 100, except in the case of the migrating cells which were measured in four samples (anterior) and five samples (posterior).

An analysis was made of the measurements at each of these stages to determine which of the three quantities, diameter, surface area or volume, would give a more normal distribution when plotted against frequency. It was found, by plotting the cumulative relative frequency against these three quantities on probability paper, that for all of the stages of development measured, the diameters gave the curve with the least skewness.[2] In fact the two vegetative amoebae curves as well as the anterior migrating cells and the spores very closely approximated the normal distribution curves, while the aggregating cells and the posterior migrating cells showed a certain skewness in the higher end of their range.

If the frequencies are plotted against diameters for all the different stages and are shown on one graph, as has been done in Figure 1, it becomes obvious that the mean size (indicated by the vertical lines) goes through considerable fluctuations during the course of development. It remains constant throughout the vegetative period but makes a dramatic drop during the aggregation stage. During the migration period, the cells increase in size, but most interesting here is the fact that the anterior presumptive stalk cells are highly significantly larger than the posterior presumptive spore cells. A spot check was made on 50 anterior and 50 posterior cells of an old migrating cell mass (one that had migrated 25 mm.) and it was found to be the same as the young cell masses tested. The spores, on the other hand, are the smallest of all the stages.

In glancing at Figure 1, one can see that the range varies for each stage and an analysis was made to see to what extent this was related to size, for the larger the mean, the larger would be the expected range if the amount of variation about the mean were the same. To compare the different stages the usual procedure of dividing the standard deviation by the mean was employed to obtain the coefficient of variation. These coefficients are indicated on Figure 1 and it can be seen·that during the first day of vegetative growth the variability is low but that it rises during the second day and remains high in the migration stage, only to drop to its lowest value in mature spores.

DISCUSSION

It is not surprising to find that the mean size does not change between one and two days of vegetative growth, and the cause of the sudden drop during aggregation is perhaps understandable for the vegetative amoebae are actively feeding and engulfing bacteria, while aggregating cells have been fasting for a considerable period of time. (In this case it has been some 17 to 18 hours since the amoebae were washed free of most of the bacteria by centrifugation.) There is no known explanation why the cells increase in size during the migration stage, but certainly it cannot involve feeding but must involve internal osmotic changes. The most important fact is that in the migrating cell mass the anterior presumptive stalk cells are significantly larger than the posterior presumptive spore cells, giving another example of an early detectable difference in differentiation. These migrating cell masses were stained with vital Nile blue sulfate and show characteristic dark anterior ends and light posterior ends (Bonner, 1952).

The variability during certain stages is remarkably high in *Dictyostelium* when one compares it to the variability of various unicellular organisms listed by Adolph (1931). To fully appreciate the consequences of this, one need only visualize the volumes; the largest two-day old vegetative amoebae, for instance, may be 18 to 19 times greater in volume than the smallest ones.

In comparing the coefficients of variation of the different stages there is an interesting trend, for the variability is low during the spore stage and rises somewhat after one day of vegetative existence, but only reaches a peak of variability after two days.

It is a curious fact that for these cell populations the diameters should give a normal distribution curve rather than the surface areas or volumes. Of course, since the factors which determine size, variability and skewness are virtually unknown, there is no reason to expect any particular type of distribution curve. An intriguing possibility might be, however, that the linear dimension gives a normal distribution because a ratio of the volume to the surface is in some way limiting to cell size and this ratio is, of course, linear. But unfortunately there is at the moment no way to weigh down such a wild speculation with a few facts.

SUMMARY

1. The individual cell size was measured for 300 cells at various stages during the development of the slime mold *Dictyostelium discoideum;* during two periods of the vegetative stage when the amoebae are actively feeding, during the aggregation stage when the amoebae are streaming together to form cell masses, during the migration stage when the cell mass wanders over the substratum, and finally the mature encapsulated spores.

2. The mean size was large during the vegetative period, dropped severely in the fasting aggregating amoebae, increased slightly during the migration stage, only to fall to their minimum size as mature spores.

3. The variability in size was large and especially so during the periods before, during and after aggregation.

4. Of special interest relative to the problem of differentiation was the fact that during the migration stage, the anterior presumptive stalk cells were significantly larger than the posterior presumptive spore cells, being another example of an early detectable sign of differentiation.

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